On Drug-Membrane Permeability of Antivirals for SARS-CoV-2

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Cite This: *J. Phys. Chem. Lett.* 2021, 12, 1384–1389

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ABSTRACT: One of the key parameters required to identify effective drugs is membrane permeability, as a compound intended for an intracellular target with poor permeability will have low efficacy. In this paper, we leverage a computational approach recently developed by our group to study the interactions between nanoparticles and mammalian membranes to study the time of entry of a variety of drugs into the viral envelope of coronavirus as well as cellular organelles. Using a combination of all-atoms molecular dynamics simulations and statistical analysis, we consider both drug characteristics and membrane properties to determine the behavior of 79 drugs and their interactions with the viral envelope, composed of the membrane and spike protein, as well as five other membranes that correspond to various mammalian compartments (lysosome, plasma, Golgi, mitochondrial, and endoplasmic reticulum membranes). The results highlight important trends that can be exploited for drug design, from the relatively high permeability of the viral envelope and the effect of transmembrane proteins, to the differences in permeability between organelles. When compared with bioavailability data present in the literature, the model results suggest a negative correlation between time of permeation and bioavailability of promising drugs. The method is general and flexible and can be employed for a variety of molecules, from small drugs to small nanoparticles, as well to a variety of biological membranes. Overall, the results indicate that this model can contribute to the identification of successful drugs as it predicts the ability of compounds to reach both intended and unintended intracellular targets.

The 2020 pandemic has brought the chasm between the rapid spread of infectious diseases and the relative slow pace of scientific discovery to stark reality. This particular event is not the first, nor it will be the last. Since the turn of the century three different coronavirus threats have surfaced, and emerging infectious diseases will continue to be a threat for the foreseeable future. Current methods for studying betacoronaviruses, responsible for the current pandemic, are technically and time demanding. Viral isolation from field sample is rarely successful, and reverse genetics recovery of recombinant virus is labor intensive and expensive. Similarly, the design and testing of new drugs is an extremely lengthy process. To this end, drug repurposing, where existing compounds that have already been tested safe in humans are redeployed to combat another disease, has emerged as a critical strategy. As an example, Remdesivir, one of the promising COVID-19 drug candidates, is a broad-spectrum drug that was originally targeted toward a number of viruses, such as SARS-CoV and MERS-CoV, but it has also been studied to counter Ebola virus infection.

Multiple characteristics define the potency of a drug, as well as its side effects. Among them, bioavailability, the fraction of a compound that reaches the intended target, is one of the hardest to estimate reliably and rapidly.¹ Although several factors contribute to defining bioavailability, passive permeability through different biological membranes is a critical

component since the inability to reach the intended target often translates into poor *in vivo* efficacy and may require higher dosages with higher risks of side effects.²

The viral lifecycle encompasses several crucial steps, starting with the attachment of the virus to the cell and finishing with the release of the progeny virions from the host cell. SARS-CoV-2 targets cells through the viral structural spike protein that binds to an enzyme on the host cell membranes. Following receptor binding, the virus uses host cell receptors and endosomes to enter cells. Once inside, viral polypeptides are synthesized that encode for the replicase-transcriptase complex. The virus then synthesizes RNA and structural proteins, leading to completion of assembly and release of viral particles. These viral lifecycle steps provide potential targets for drug therapy, and most of the drugs currently in clinical trials inhibit key components of the infection lifecycle, such as viral entry into the host cell, viral replication, and viral RNA synthesis.⁴ These targets are localized to particular subcellular compartments, yet current drug design strategies are focused

Received: August 5, 2020

Accepted: January 12, 2021

Figure 1. Methods and data source used in this work.

on general bioavailability and rarely address drug delivery to specific tissues or intracellular compartments. Therefore, knowledge of the permeability of antiviral drugs, with reference to both viral and mammalian cell compartments, is vitally important from both a pharmacokinetics and drug design standpoint. In an effort to accelerate translation of promising drug candidates, as well as to eliminate potentially ineffective or even harmful candidates, we present a science-based model to predict the permeation of drug candidates for SARS-CoV-2 into coronavirus membrane and mammalian membranes.

Over the past decades many models have been developed to estimate the permeability of drugs. The most commonly used ones are the empirical “rule of five”,⁵ QSAR (quantitative structure–activity relationship),⁶ and QSPR (quantitative structure–property relationship) methods.⁷ While QSAR/QSPR methods have shown their efficacy in screening the binding of antiviral drugs and targets, the predicted permeability is at best only accurate to an order of magnitude, mostly due to the incorporation of too many descriptors that are not related to the permeation process and the strong dependence on the quality of the training sets employed.⁸ On the other end of the accuracy spectrum, several methods have been proposed over the years that leverage a physical description of the passive permeation process, like the inhomogeneous solubility diffusion model and state-transition model.¹ These models, which currently often involve biased molecular dynamics (MD) simulations, are either computationally too expensive for a rapid application or suffer from limited accuracy when drugs of various sizes and shapes or membranes with different lipid profiles are involved.

To bridge the gap between accuracy and ease of use, we have recently introduced a model to predict the rates of passive permeation of molecules and nanoparticles (up to several nanometers) into phosphatidylcholine membranes.⁹ The low-density area (LDA) model combines five parameters, three of them that describe the permeant (i.e., size/shape, diffusivity, and hydrophobicity) and two that describe the membrane (i.e., the size and lifetime of the low-density regions on the membrane surface) to compute the average time required for the permeant to enter a membrane. The time of entry can then be related to the average speed of crossing the membrane; more details can be found in the Supporting Information and the original work.⁹ The LDA model, which can combine both experimental and computational data, has shown accuracy equivalent to fully atomistic MD methods with a much smaller computational cost. Such a result was achieved observing that

the permeation process of small compounds is controlled by the formation of low-density areas on the membrane surface and by factorizing the permeation process into independent contributions of the membrane thermal and pressure fluctuations and characteristics of the molecules (size, shape, and lipophilicity).

Leveraging the LDA model, here we report on the permeation times of promising drugs candidates through coronavirus membranes, with the overarching goal of providing inputs to help the design and selection of new or repurposed drugs. Additionally, since the targets of many drugs are located inside mammalian cells, we also report on the permeation kinetics of the same drugs through five mammalian membranes. A schematic of the overall approach and data sources used in this study is shown in Figure 1. The estimates were obtained using a combination of molecular dynamics simulations in conjunction with an improved LDA model for membranes (refer to the methodology section in the Supporting Information, drug properties for the improvements). Seventy-nine drugs for SARS-CoV-2 were analyzed, comprising FDA-approved drugs, compounds in clinical trials, and preclinical compounds.⁴ The computational results are compared with data available in the literature on bioavailability and show the potential of the model to aid in the assessment of drugs’ permeability into a variety of organelles.

Coronaviridae is a family of large, enveloped, positive-stranded RNA viruses. The envelope is derived from the host endoplasmic reticulum (ER) membrane and consists of a lipid bilayer, in which structural proteins are anchored. The surface glycoprotein spike that forms large protrusions from the virus surface binds to the host-cell receptor and mediates viral entry.^{10,11} Since surface characteristics of the biological membranes determine the permeability of drugs, we developed an atomistic model to describe the anchoring of the spike protein to the lipid bilayer.

The spike contains three segments, namely, a large ectodomain, a single-pass transmembrane anchor, and a short intracellular tail. The region outside the viral core forms alpha helices where coiled-coil interactions, through hydrophobic contact, help stabilize the chains. The tails near the C-terminal are mostly polar with charged residues that form random coils inside the viral core (i.e., the cytoplasmic side after membrane fusion with the host cell); interactions between the charged residues and lipid head groups stabilize the end of the transmembrane region of the spike protein. A diagram of a section of the coronavirus envelope composed of

Figure 2. Structural diagram of the coronavirus membrane with the anchored COVID-19 spike protein. (A) Protein colored according to secondary structure (purple for α helix, red for β sheet, and gray for random coils). In the lipid bilayer, carbon atoms are shown in gray, nitrogen in red, oxygen in blue, and phosphate in tan; cholesterol is shown uniformly in orange. Hydrogen atoms are omitted for clarity. (B) The anchoring region (part of the heptad repeated, the transmembrane region, and the C-tails) of the spike protein in the membrane. (C) The anchoring region of the spike protein colored by the residue type. Nonpolar residues are colored in white, while polar residues are in green. Images generated with VMD.³

lipid bilayer and the anchored spike protein is shown in Figure 2.

On the basis of the existing data and estimated secondary structure,^{12,13} we assumed that the transmembrane region of the COVID-19 spike protein is formed by the residues in the #1210–1235 range. The charge–charge interactions among residues 1215:TYR, 1219:GLY, and 1223:GLY contribute to holding the three chains together inside the hydrophobic region of the membrane lipids.

To test our hypothesis, we run MD simulations of the spike protein anchored to a lipid bilayer with a composition similar to the one of the ER membrane, from which coronaviruses acquire their membrane envelopes, but with a slightly higher concentrations of sphingomyelin and phosphatidylinositol.¹⁴ As we will show later, this slight change in composition results in an observable difference in the time of entry of the various compounds.

The density of the spike protein used in our simulations approximates the experimental average area of about 57 nm² to 101 nm² per transmembrane protein, estimated from a viral spherical envelop with a radius of 60 to 80 nm¹⁵ and an approximate number of 800 transmembrane proteins per virion.¹⁶ To reduce the computational cost, after the initial equilibration run used to validate the stability of the protein anchoring region, we considered only part of the protein (Figure 2B and C) as the rest of the spike protein is far enough away (5 nm or more) to not directly affect the formation and lifetime of low-density areas on the viral membrane surface.

In addition to the viral envelope described before (with and without spike protein), we studied the surface properties of five membranes corresponding to various mammalian compartments (lysosome, plasma, Golgi, mitochondrial, and endoplasmic reticulum membranes) using all-atom molecular dynamics simulations.

Figure 3 reports the distributions of the permeation times of 79 drugs in various membranes relative to the ER membrane,

Figure 3. Times of entry ratios of 79 drugs in different membranes relative to the ER membrane.

which was chosen as a reference since the permeation in this membrane is the fastest for all drugs. The results show that membrane composition and the presence of transmembrane proteins noticeably affect the permeability of the membrane.

As expected, lysosome and plasma membranes show longer times of entry than the other mammalian membranes. Differently, the viral membrane is more permeable, although the inclusion of the transmembrane proteins has a noticeable impact. When comparing the distribution of permeation times

Figure 4. Times of permeation for drugs candidates in different organelle membranes, grouped by (A) viral baits and (B) related mammalian processes when viral baits were unknown. Error bars represent one standard deviation. Tabulated data in the [Supporting Information](#).

for ER and coronavirus membrane, with and without the spike protein, we observe a $\sim 40\%$ increase in the permeation time due to the presence of proteins and $\sim 10\%$ due to the change in lipid composition. Likely, the presence of proteins affect the distributions and lifetimes of the low-density areas due to the reduced mobility of the lipids near a rigid object, similar to what has been observed for C_{60} fullerenes in lipid bilayers.¹⁷ While the presence of the spike protein in the coronavirus membrane results in an increased stiffness, the viral membrane is still generally two times more permeable than the plasma membrane and lysosomes. Although currently very few drugs are required to cross viral membranes, this difference can

potentially be exploited by other classes of compounds, like nanoparticles, which can amplify the effect of membrane stiffness due to their relative large size.

The individual times of entry of drug candidates grouped by viral baits or, when that is unknown, by biological processes, are shown in [Figure 4](#). The results show a variety of time scales going from nanoseconds to microseconds depending on the drug and membrane. Albeit the bioavailability of a drug is affected by many factors, like metabolism rate and plasma protein binding,¹⁸ we find a negative correlation between the bioavailability of drugs and the permeation time predicted by the LDA model. For example, for drugs within the same group,

Lisinopril (Figure 4B, Cell Entry) is the least permeable compound as well as the compound with the lowest bioavailability (25% based on experimental data¹⁹). On the other hand, Captopril, Nafamostat, and Camostat, for which we predict permeation times that are one order of magnitude shorter than Lisinopril, have an estimated bioavailability of 100% (data from <http://drugbank.ca>). Similar observations can be made for Rapamycin (Figure 4A, N), which has lower bioavailability than Sapanisertib and Silmitasertib. While undeniably other factors affect the ability of a drug to reach the intended target, these results seem to indicate that the target membrane permeability can play an important role in bioavailability.

Interestingly, the entry times of drugs that are currently identified as the most promising candidates against COVID-19 during their clinical trial²⁰ are among the shortest compared to other drugs with similar targets. For example, drugs related to the disruption of viral translation such as Ternatin-4²¹ (Figure 4B Translation), Zotatfin,²² (Figure 4A, Nsp2), and Plitidepsin²³ (Figure 4B, Translation); sigma receptors such as Haloperidol²⁴ and Compound PB28²⁵ (Figure 4A Nsp6 and Orf9c); Melperone, Cloperastine,²⁶ and Clemastine (Figure 4B, Protein Processing); and the female hormone progesterone (Figure 4B, Viral transcription) are among the most permeable compounds in their respective categories.

In summary, in this paper we have used the LDA model to compute the times of entry of drugs that target various steps in the lifecycle of infection of SARS-CoV-2. The model is based on the LDA theory previously developed by our group, which describes the permeation process as regulated by the low-density area on the membrane surface and the size, shape, and solubility of drugs in the hydrophobic phase of the membrane itself. Starting from available experimental data on SARS-CoV-2, we developed an atomistic model of the spike protein anchored to the lipid bilayer to study the viral envelope using molecular dynamics simulations. Since antivirals target different stages in the lifetime of a virus, we determined the times of entry of drugs in the viral envelope and five mammalian membranes to determine the dynamics and characteristics of the membranes. The results show several noteworthy trends, from the relatively high permeability of the viral envelope to the differences in permeability between organelles. Also, the times of entry, which can be related to the average time of permeation, when compared with the limited bioavailability data present in the literature, show a general negative correlation, despite the large number of extraneous factors that affect the bioavailability.

Overall, the results highlight how the LDA model can be applied to predict the average time of crossing of drugs through various membranes and how researchers can gain insights on the effect of differences between membranes of different organs or organisms. From a drug design, as well as health assessment perspective, these predictive capabilities can facilitate compound evaluation by ruling out candidates with low permeability or excessive permeability in unintended organs or organelles. Indeed, the LDA model can be used to estimate the absorption selectivity of different targets (e.g., carcinogenic cells, organs) by molecules and nanoparticles without need of a large set of preexisting data, making it an invaluable tool for the comprehensive and fast validation pipeline of new compounds. As such, while the results reported in this paper focus on drugs for SARS-CoV-2, the method is

general and flexible and can be employed with other tools to shorten the effective response time to a global pandemic.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at <https://pubs.acs.org/doi/10.1021/acs.jpclett.0c02397>.

Additional information about the methods, drugs, and membranes used in this study (PDF)

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Notes

Data Availability: Time of permeation of drugs and the lipid profile of membrane generated during this study are included in this published article (and its Supporting Information files). The MD simulation trajectories generated during and/or analyzed during the current study are in total 379 GB in size and are available from the corresponding author upon reasonable request.

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

The authors are grateful to Dr. Matteo Masetti and Dr. J. Scott VanEpps for the discussions and suggestions. This work was supported by the University of Michigan, BlueSky project.

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